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Impact of irradiance on the epidermis of *Impatiens flanaganae* Hemsl.

N. Lall and R.B. Bhat*

Department of Botany, University of Transkei, Private Bag X1, Umtata, 5100 Republic of South Africa

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Variations in the epidermis of *Impatiens flanaganae* Hemsl. grown under different light conditions were investigated. The difference in light intensity triggered an extrinsic and intrinsic instability which greatly promoted aberrant stomatal development and variations of epidermal cells. Four types of normal stomata were observed. The ontogeny of stomata was either perigenous or mesogenous. An increase in stomatal abnormalities was noticed in leaves subjected to greater and lower light intensities than in the control. Abnormalities such as degeneration of guard cells, superimposed and juxtaposed contiguous stomata, stomata with persistent intervening walls, unequal guard cells, cytoplasmic connections, single guard cells, guard cells without pores, and persistent stomatal cells were noticed. The size and shape of epidermal cells, stomatal index, frequency of stomata and epidermal cells were also compared and recorded in different variants. These results have implications for the conservation of this endangered species.

Keywords: Conservation, epidermal structure, *Impatiens flanaganae*, radiation, stomata.

*To whom correspondence should be addressed.

Introduction

The Balsaminaceae is a bigeneric family consisting of herbs (rarely subshrubby) with subsucculent, nearly always glabrous stems, with 850 species, mainly distributed in the tropics with a few in temperate regions (Mabberley 1987). *Impatiens flanaganae* Hemsl. is known only from a few locations in southern KwaZulu-Natal and the Eastern Cape of South Africa, where it is endemic. Within the family Balsaminaceae, the monotypic genus *Hydrocera* and the prolific genus *Impatiens* are recognized and commonly referred to as Balsams. A literature review reveals that very little work has been carried out on a few species only of *Impatiens*. Protein accumulation during maturation of the pods of *I. balsamina* L. has been reported by Pal and Biswas (1994). Schulz *et al.* (1993) investigated gas exchange of *I. pallida* Nutt. in relation to wilting under high irradiance. Post-harvest quality of potted *I. walleriana* Hook. f. ex. D. Oliver as influenced by silver thiosulphate application and light conditions was investigated by Doi *et al.* (1992). Schmitt (1993) studied reaction norms of morphological and life-history traits to light availability in *I. capensis* Thunb. The effect of temperature and photoperiod on the growth and flowering in New Guinea *Impatiens* hybrids was described by Yamauchi *et al.* (1991). Recently Lall and Bhat (1996) investigated the normal and abnormal stomatal development in *I. flanaganae*. No other work has been recorded on this species. As per Transkeian Nature Conservation Act 1971, *I. flanaganae* is grouped under Schedule 5 of Endangered flora. It is a tuberous, rooted perennial with a tall, fleshy, unbranched stem which can grow up to 1.5 m in height in moist shady conditions. In nature, the tubers of *I. flanaganae* start sprouting in September and generally flower between December and March. After flowering, the plant dies off, leaving tubers that remain dormant in the ground for up to six months. These tubers are used as food by ground animals or in the preparation of cough mixture by the indigenous people. Moreover, the bright pink flowers of *I. flanaganae* make them attractive to horticulturists (Grey-Wilson 1980). In their natural habitat, the population size of this species is inversely proportional to light intensity. The aim of this article was to investigate the effect of light conditions on the epidermis and the importance of light intensity to the survival of this endangered species.

Materials and Methods

Tubers were collected from the Port St. Johns area of the Eastern Cape and grown to maturity in controlled laboratory conditions. Fresh leaves from natural populations grown under different light intensities and from plants grown under various light conditions (mercury vapour lamps) in growth rooms were collected for the epidermal studies (see Table 1). The light intensity was measured using the Crump quantum: radiometre: photometer. In natural populations, larger numbers of healthy plants were found growing under the light intensity of 5.5 W m^{-2}, which was taken as control. An acetic acid solution (30%) was used to test the type of calcium crystals present in the leaves. Epidermal peels taken from the adaxial and abaxial surfaces of the leaves of all the variants were stained with Delafield's haematoxylin and mounted in glycerine. The technique of Bhat and Etejere (1985) was also used to take imprints of the epidermis. Mean values of 30 observations showing stomatal frequency, stomatal

Table 1 Variants of *Impatiens flanaganae* grown under different light conditions

Variant no.	Light intensity (Wm^{-2})	Remarks
1	0.5	Shade – growth room
2	3.0	Shade – growth room
3	5.5	Control
4	8.0	Natural population
5	10.5	Natural population
6	13	Natural population
7	15.5	Natural population
8	18	Natural population
9	20.5	Natural population
10	23.0	Natural population
11	25.5	Growth room
12	28.0	Growth room
13	30.5	Growth room

Table 2 Frequency of stomata and epidermal cells per mm² index of stomata per mm² and size of guard and epidermal cells in µm in the lower leaves of *Impatiens flanaganae*

		Stomata				Epidermis		
	Variants light intensity	Frequency (no. of stomata		Size of guard cells in µm		Frequency (no. of epidermal	Size of epidermal cells in µm	
Variants	(W m^{-2})	mm^{-2})	Index	L_l	B	cells mm^{-2})	L_l	B
V1	0.5	10 ± 0.94	13	43 ± 0.96	12 ± 1.07	66 ± 1.00	83 ± 0.91	58 ± 1.07
V2	3	12 ± 1.28	13	32 ± 0.97	10 ± 1.03	78 ± 0.92	70 ± 0.93	55 ± 1.03
V3*	5.5	13 ± 1.13	14	24 ± 0.73	10 ± 0.76	80 ± 0.91	63 ± 0.73	54 ± 0.77
V4	8	13 ± 1.10	14	24 ± 0.70	11 ± 1.50	80 ± 1.25	64 ± 0.79	55 ± 1.45
V5	10.5	14 ± 0.95	15	24 ± 1.03	12 ± 1.00	82 ± 1.10	65 ± 1.03	54 ± 1.00
V6	13	15 ± 0.91	15	25 ± 0.78	12 ± 1.26	83 ± 1.03	65 ± 0.78	54 ± 1.26
V7	15.5	15 ± 1.00	15	25 ± 0.82	12 ± 1.15	84 ± 0.91	64 ± 0.82	53 ± 1.15
V8	18	16 ± O.96	16	25 ± 0.84	13 ± 1.26	84 ± 1.13	64 ± 0.84	53 ± 1.28
V9	20.5	16 ± 0.84	16	25 ± 0.90	13 ± 1.08	83 ± 1.24	64 ± 0.96	54 ± 1.08
V10	23	17 ± 0.73	17	26 ± 0.96	12 ± 1.13	83 ± 1.25	65 ± 0.90	52 ± 1.13
V11	25.5	17 ± 0.95	17	26 ± 1.08	12 ± 1.31	83 ± 0.66	64 ± 1.08	52 ± 1.31
V12	28	18 ± 0.91	18	27 ± 1.15	11 ± 1.51	84 ± 0.99	63 ± 1.15	52 ± 1.50
V13	30.5	18 ± 0.82	18	27 ± 0.93	10 ± 1.35	84 ± 091	63 ± 0.95	53 ± 1.31

V = variant, L_l = length, B = breadth, * = control

index, frequency of epidermal cells and size of guard and epidermal cells are compiled in Table 2. Observations have been supported by *camera lucida* drawings, made using a Leitz Lab 11 microscope, at constant magnification.

Results

Epidermis

The epidermal cells were elongated with sinuous anticlinal walls (Figures 2–12). The leaves were hypostomatic and stomata were distributed all over the abaxial surface, except over the veins, without any definite pattern of orientation. Bundles of calcium carbonate crystals were observed in a few epidermal cells (Figure 10). Unicellular and uniseriate multicellular trichomes were rarely observed on the mature leaves (Figures 2 & 3). The trichomes were generally caducous in nature.

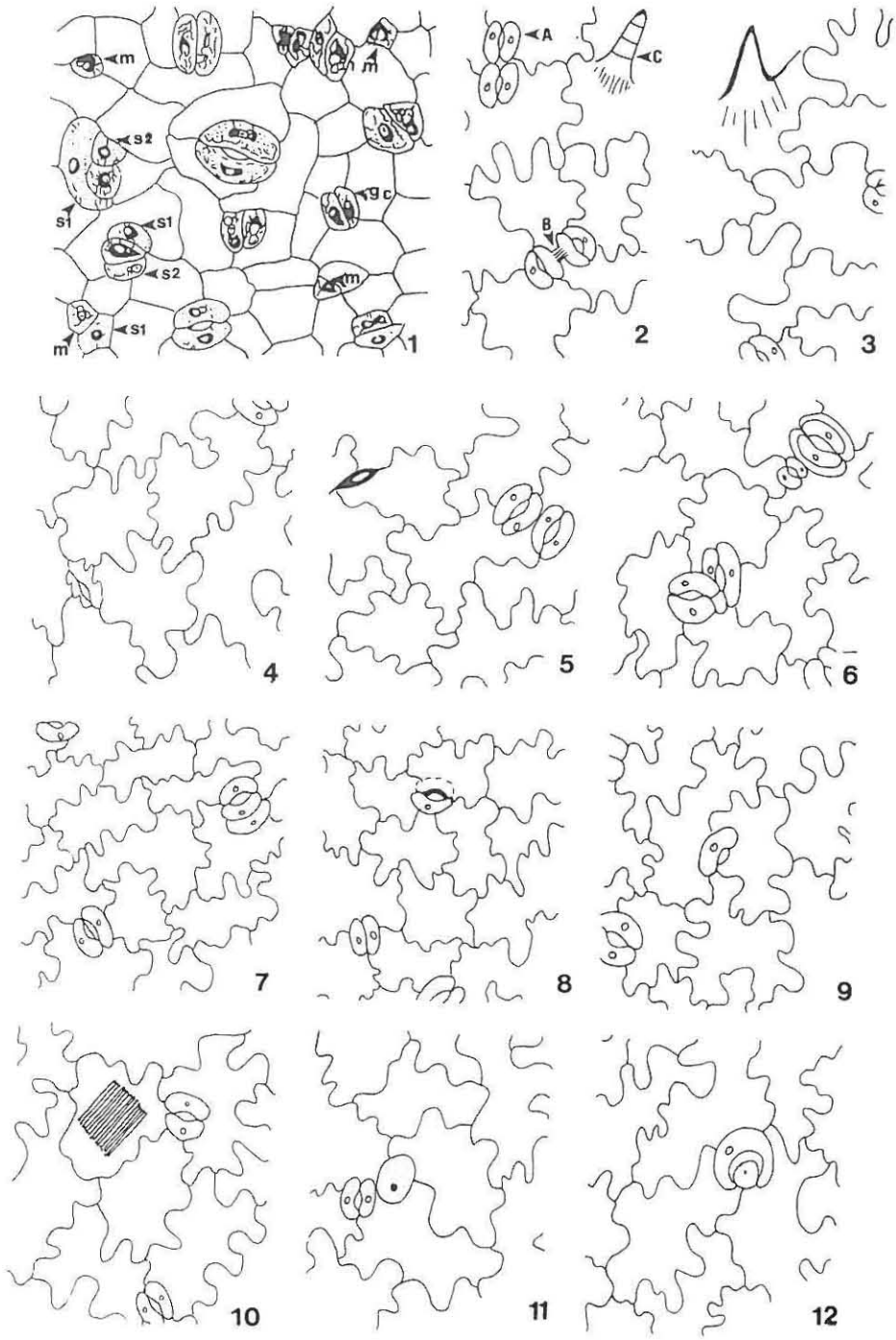
Stomata

Anomocytic, anisocytic and stomata with single subsidiary cells were the stomatal types observed in almost all the variants, whereas only the paracytic type was observed in variants 11, 12 and 13. The anomocytic stomata of Metcalte and Chalk (1950) were the predominant type observed. These are surrounded by 3–6 ordinary epidermal cells (Figures 6 & 8). Anisocytic stomata are surrounded by three subsidiary cells, of which one is distinctly smaller than the other two (Figures 7 & 9). Paracytic stomata are flanked by two parallel and lateral subsidiary cells which are either non-contiguous or contiguous at one or both of the poles (Figure 6). Stomata with a single subsidiary cell were also present (Figure 7).

Ontogeny of stomata

The protoderm cells were uninucleate, polygonal, isodiametric, with straight or slightly arched walls and uniform staining properties. A meristemoid is cut off from any protoderm cell, either in a corner or on one side. The meristemoid can easily be distinguished by its shape, smaller size, prominent nucleus and differential staining. Meristemoids were either solitary or in pairs (Figure 1). The development of different types of stomata was as follows:

1. Anomocytic: the meristemoid directly functioned as a guard mother cell without cutting off any subsidiary cells. It enlarged, became rounded and divided by a straight wall to form a pair of guard cells (Figure 1). A lenticular pore developed in between the two guard cells.
2. Anisocytic: the meristemoid divided by a slightly curved wall to form two unequal cells. The larger cell differentiated as the first subsidiary cell while the smaller one enlarged and divided by producing a slightly curved wall perpendicular to the first, to give rise to the second cell and a middle cell. The middle cell enlarged, divided by forming a curved wall intersecting the first and second, to form the third subsidiary cell and a central guard mother cell. The guard mother cell divided by forming a straight wall to produce two guard cells (Figure 1). Here, the meristemoid behaved like an apical cell with three cutting faces.
3. Paracytic: the meristemoid cut off two parallel subsidiary cells and then functioned as the guard mother cell, which divided vertically by producing a straight wall parallel to the subsidiaries, to give rise to two equal guard cells (Figure 1). The two subsidiary cells flanking the guard cells were either non-contiguous or contiguous at one or both of the poles, depending upon the non-intersection or intersection of the walls.
4. Stomata with a single subsidiary cell: the meristemoid divided by forming a slightly curved wall, to produce two unequal cells, of which the larger differentiated as a single subsidiary cell while the smaller one functioned as a guard mother cell. The guard mother cell divided by a straight wall to produce a pair of guard cells.



Figures 1–12 **1.** Stages in development of stomata. **2.** (A) Superimposed contiguous stomata, (B) cytoplasmic connection between the stomata, (C) multicellular uniseriate trichome. **3.** Unicellular trichome. **4.** Arrested development of stomata. **5.** Degeneration of guard cells, juxtaposed contiguous stomata. **6.** Paracytic stoma, and contiguous anomocytic stomata at right angles. **7.** Anisocytic stoma, stoma with a single subsidiary cell, and stomata with a persistent intervening wall in the stomatal pore. **8.** Anomocytic stoma, degeneration of guard cell, guard cells without pore. **9.** Anisocytic stoma, single guard cell. **10.** Calcium carbonate crystals 'raphides'. **11.** Persistent stomatal cell contiguous with normal stoma. **12.** Unequal guard cells. Magnification of Figure 1 × 600; 2–12 × 250; m = meristemoid, gc = guard cells, sl = subsidiary cell one; s2 = subsidiary cell two.

Stomatal aberrations were commonly observed in low and high light intensities (Figure 13). Aberrant types observed in the variants were: (l) arrested development, (2) unequal guard cells, (3) single guard cells, (4) degeneration of guard cells, (5) persistent stomatal cells, (6) cytoplasmic connections, (7) guard cells without pore, (8) contiguous stomata and (9) stomata with persistent intervening wall.

1. Arrested development: during ontogeny the stomatal development got arrested at a meristemoid stage after the cutting off of one to several subsidiary cells at guard mother cell stage, or after the formation of guard cells. In the stages of arrested development, the cytoplasm became vacuolated and degeneration of the nucleus was followed by that of the cytoplasm. These stages remained *in situ* but looked like epidermal cells (Figure 4). Such arrested developments were observed in V1 and V2 in our study.
2. Unequal guard cells: the meristemoid normally divided to form two equal guard cells, but sometimes it produced two

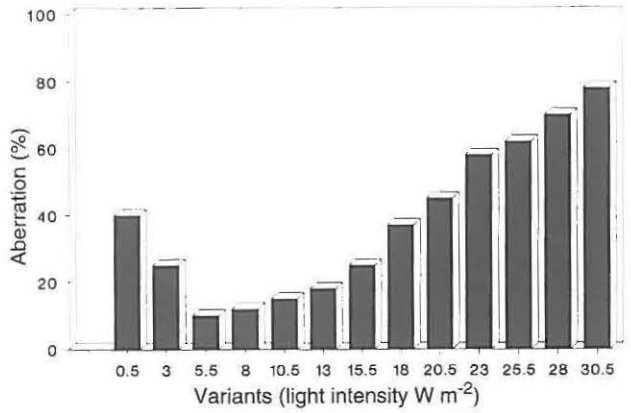


Figure 13 Stomatal aberrations of *I. flanaganae*

unequal ones. Rarely, one of the guard cells enlarged and encroached rapidly upon the other. Sometimes the meristemoid underwent a ring-shaped division, resulting in two unequal guard cells, the larger cell encircling the smaller one except on the side where it was exposed (Figure 12). Unequal guard cells were observed in V2, V11 and V12.

3. Single guard cells: these arose in three ways. (a) Directly from the meristemoid, where the guard mother cell did not divide but enlarged, developed notches on one side and a differential thickening appeared in the notch region. A pore might develop; (b) as a result of degeneration of one of the guard cells before or after the pore formation (Figure 9); (c) by differentiation of one of the guard cells into an epidermal cell. Single guard cells were observed in V1, V2, V6 and V7.
4. Degeneration of guard cells: one or both of the guard cells of a stoma became aborted due to degeneration. First, the guard cells developed thin walls, the cytoplasm became highly vacuolated and the nucleus degenerated, followed by the cytoplasm and cell contents. The adjacent surrounding epidermal cell encroached upon the degenerated guard cell and ultimately only a thickening was left around the pore (Figures 5 & 8). This was observed in V2, V3, V9, V10 and V11.
5. Persistent stomatal cells: The meristemoid either directly differentiated as a persistent stomatal cell or it cut off one or more subsidiary cells which formed a persistent stomatal cell. In the former case, the meristemoid did not divide, it enlarged, the wall became uniformly thickened and chloroplasts appeared within the cell (Figure 11). Persistent cells were spherical in shape and they differed from the meristemoid by the presence of distinct chloroplasts and by their staining properties, which were similar to those of the guard cells. They differed from guard cells in the lack of differential thickening. They were noticed in V7, V12 and V13.
6. Cytoplasmic connection: the guard cells of the neighhouring stomata were sometimes connected by a cytoplasmic band (Figure 2). Thus the protoplasts of the guard cells of two neighbouring stomata seemed to be in communication. These connections may serve as pathways for the translocation of materials between the guard cells of the two stomata. Such connections were observed in V7, V8, V11 and V12.
7. Guard cells without pores: sometimes the meristemoid divided normally to produce two equal guard cells but the pore between them did not develop (Figure 8). Such guard cells without pores were found in V1, V9 and V10.
8. Contiguous stomata: these developed from adjacent meristemoids. Contiguous stomata were variously oriented and their orientation depended on the plane of division in the adjacent guard mother cells. Contiguous stomata were superimposed, juxtaposed, or at right angles to each other (Figures 2, 5 & 6). Contiguous stomata were observed in V2, V7, V8, V11 & V12.
9. Stomata with a persistent intervening wall: the presence of a persistent intervening wall in some of the stomatal cavities was a unique feature in the species (Figure 7) and was observed in V8 and V9.

Discussion and Conclusion

Kropfitsch (1951a, 1951b) studied the effect of ultraviolet light and apple gas on stomata. Inamdar *et al.* (1974) described the effect of gamma radiation and other factors on the stomata of *Brassica junceae*, and reported the occurrence of persistent stomatal cells in the irradiated plants. Our results conformed to those of Inamdar *et al.* (1974). Persistent stomatal cells were observed in the variants subjected to light intensities higher than 15 V m^{-2}. The presence of a persistent stomatal cell contiguous with the normal stoma may reveal that a guard mother cell is capable of further division to form two daughter guard mother cells, which may develop into two contiguous stomata. In the observed case, one of the daughter guard mother cells would have remained persistent.

During this study, the following stomatal types were observed: anomocytic, anisocytic, paracytic and stomata with a single subsidiary cell. The anomocytic stomata were predominant over the other types. The ontogeny of anomocytic stomata conformed to that of the perigenous type of Pant (1965) while that of the other types conformed to the mesogenous type of Pant (1965).

With the gradual increase in light intensity, there was a corresponding increase in the stomatal frequency and the stomatal index in *I. flanaganae*. However Inamdar *et al.* (1974) reported in *Brassica junceae* L. a decrease in stomatal index and stomatal frequency as the dose of gamma irradiation increased. Under low light intensity (< 5 W m^{-2}), the size of guard and epidermal cells was larger than that of the other variants, which may have been due to an etiolation effect. It was noticed that the gradual increase in light intensity led to some morphological expressions such as reduction in leaf size, short internodes and pale green to pale yellow leaves. Our observation was in agreement with the report of Inamdar *et al.* (1975), where it was pointed out that the external relative difference expressed internal reduction of tissues in *Catharanthus roseus* (L.) G.Don f. infected by little leaf virus

The aberrant stomatal types noticed were: persistent stomatal cells, arrested development, unequal guard cells, single guard cells, cytoplasmic connections between nearby stomata, guard cells without pores, and contiguous stomata. The presence of a persistent intervening wall in some of the stomatal cavities was observed in some variants. This may have been due to lack of sufficient pectinase production in the guard cells which is responsible for dissolving the middle lamella. The formation of contiguous stomata may be due to the development of adjacent meristemoids or to the readjustment of epidermal cells and stomata during maturation. This conformed to the view of Bunning (1952). Contiguous stomata could also be formed as a result of the division of a guard mother cell into two daughter guard mother cells, which could develop into two contiguous stomata. Similar observations have been made by Samuel and Bhat (1994) in *Stenoglottis fimbriata* Lindl. In variants subjected to higher light intensity, the formation of contiguous stomata may have been due to degeneration and disintegration of the anticlinal walls that radiate from the guard cells, resulting in the drift of the guard cell/s to the neighbouring stoma. Drifting stomata may also result in the formation of stomata that are juxtaposed, superimposed or at right angles based on the position of the guard cells.

Several explanations have been offered regarding the cause of aberrant stomatal formations. According to Morgan (1934) stomatal aberrations may be due to cytoplasmic heterogeneity, while McClintock (1956) attributed them to gene action. Bunning (1952) suggested that aberrations are due to extrinsic factors. Inamdar *et al.* (1977) reported that gamma irradiation caused a number of stomatal aberrations. According to Percy and Riding (1978), Swiecki *et al.* (1982) and Percy (1985), certain air pollutants and acid rain can interfere with the development of the cuticle and wax and cause significant damage to the epidermis and interior tissues. Samuel and Bhat (1994) suggested that some extrinsic factors such as radiation pathogens and even pollutants may be able to trigger an intrinsic instability which may finally lead to aberrations of epidermal tissues.

Although in *I. flanaganae* abnormalities occur both in normal as well as in plants kept under high or low light intensities, the frequency is higher in plants subjected to high or low light intensities. The present investigation indicates that a light intensity of less than 3 W m^{-2} and greater than 10 W m^{-2} may be lethal, whereas a light intensity between 4 and 9 W m^{-2} seems to be most favourable for the survival of the plant. The minimum of stomatal aberrations observed at this range supports our view. The present study leads one to conclude that deforestation and removal of the forest canopy, which subjects this species to excessive sunlight, may challenge its survival. Further investigations are necessary to reach a clear conclusion with regard to the environmental factors which may contribute to the conservation of this endangered species.

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